# Chromosomal Locations of *mcr-1* and $bla_{CTX-M-15}$ in Fluoroquinolone-Resistant *Escherichia coli* ST410

**Linda Falgenhauer, Said-Elias Waezsada, Konrad Gwozdzinski, Hiren Ghosh, Swapnil Doijad, Boyke Bunk, Cathrin Spröer, Can Imirzalioglu, Harald Seifert, Alexandra Irrgang, Jennie Fischer, Beatriz Guerra,[1] Annemarie Käsbohrer, Jörg Overmann, Alexander Goesmann, Trinad Chakraborty**

Author affiliations: Justus Liebig University, Giessen, Germany (L. Falgenhauer, S.-E. Waezsada, K. Gwozdzinski, H. Ghosh, S. Doijad, C. Imirzalioglu, A. Goesmann, T. Chakraborty); German Center for Infection Research, Giessen, Germany (L. Falgenhauer, S.-E. Waezsada, K. Gwozdzinski, H. Ghosh, S. Doijad, C. Imirzalioglu, T. Chakraborty); Leibniz Institute DSMZ—German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany (B. Bunk, C. Spröer, J. Overmann); German Center for Infection Research, Braunschweig (B. Bunk, C. Spröer, J. Overmann); University of Cologne, Cologne, Germany (H. Seifert); German Center for Infection Research, Cologne (H. Seifert); Federal Institute for Risk Assessment, Berlin, Germany (A. Irrgang, J. Fischer, B. Guerra, A. Käsbohrer)

DOI: http://dx.doi.org/10.3201/eid2209.160692

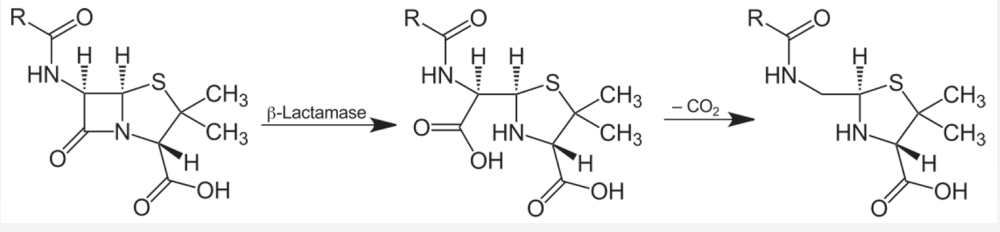
**To the Editor:** Recently, Yi-Yun Liu et al. reported on the discovery of *mcr-1*, a plasmidborne resistance gene mediating resistance to colistin, in isolates obtained from humans and animals (*1*). Since the original publication, *mcr-1* with or without the insertion element IS*Apl1* has been detected on plasmids of different incompatibility groups, including IncI2, IncHI2, and IncX4, and in many different countries (*1–3*). Because colistin is a last-resort parenteral antimicrobial drug, the transfer of *mcr-1* by

[1]Current address: European Food Safety Authority, Parma, Italy.

# etymologia

## β-Lactamase [ba'tə lak'tə-mās]

Enzymes that catalyze the cleavage of β-lactam rings in penicillins, cephalosporins, monobactams, and carbapenems were first described by Abraham and Chain in 1940. These enzymes confer resistance to β-lactam antibiotics on bacteria that produce them. β-lactamases are ancient, theorized to have evolved 1–2 billion years ago, but the emergence and spread of penicillin-resistant staphylococci in hospitals in the 1950s showed how penicillin use could select producers from a population of nonproducers. "Lactam" is a portmanteau of "**lac**tone" (from the Latin *lactis*, "milk," since lactic acid was isolated from soured milk) and "**am**ide." The "β" refers to the nitrogen's position on the second carbon in the ring. The suffix "-ase," indicating an enzyme, is derived from "diastase" (from the Greek *diastasis*, "separation"), the first enzyme discovered in 1833 by Payen and Persoz.




Action of β-lactamase and decarboxylation of the β-lactam ring. Equation by Jü, own work, public domain, https://commons.wikimedia.org/w/index.php?curid=11204303

### Sources

1. Abraham EP. A retrospective review of β-lactamases. J Chemother. 1991;3:67–74.
2. Dixon M. The history of enzymes and of biological oxidations. In: Needham J, editor. The chemistry of life: lectures on the history of biochemistry. London: Cambridge University Press; 1970. p. 15–37.
3. Dorland's illustrated medical dictionary. 32nd ed. Philadelphia: Elsevier Saunders; 2012.

Address for correspondence: Ronnie Henry, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop E03, Atlanta, GA 30329-4027, USA; email: boq3@cdc.gov

DOI: http://dx.doi.org/10.3201/eid2209.ET2209

conjugation or through mobilizable plasmids raises concern about the emergence of pan-resistant *Enterobacteriaceae*.

We previously described extended-spectrum β-lactamase (ESBL)–producing and carbapenemase-producing isolates obtained from livestock and a human in Germany that harbored the *mcr-1* gene (*2*). Because the transfer of *mcr-1* through the food chain is highly likely, we looked for its presence in 62 whole-genome sequenced ESBL-producing *Escherichia coli* isolates obtained during 2012–2013 from food products sampled in Germany (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/8/16-0692-Techapp1.pdf). We detected 4 isolates harboring the *mcr-1* gene (*E. coli* RL138, RL145, RL158, and RL465) that displayed a colistin MIC of 4 mg/L (online Technical Appendix Table 1). The raw sequencing reads and the assembled contigs of the *mcr-1*–positive isolates were deposited in the European Nucleotide Archive under project accession no. PRJEB13470. We conducted conjugation experiments to analyze the transferability of *mcr-1* (online Technical Appendix). For all isolates except RL465, *mcr-1* was transferable to *E. coli* J53 *Az*$^{r}$. For isolates RL138, RL145, and RL158, the *mcr-1* gene was present on IncX4 and IncHI2 plasmids (Figure, panel A, http://wwwnc.cdc.gov/EID/article/22/8/16-0692-F1.htm; online Technical Appendix Table 2). The sequence type (ST) 410 *E. coli* isolate RL465 was detected in a turkey hen meat sample from 2013 and harbored $bla_{\text{CTX-M-15}}$ and *mcr-1*, a gene combination hitherto identified only in travelers from the Netherlands and children from China (*4*). Both the $bla_{\text{CTX-M-15}}$ and *mcr-1* genes were not transferable, indicating that neither gene was plasmid-encoded. Examination of the genetic environment of *mcr-1* in the assembled gapped genome showed a chromosomal location for the *mcr-1* transposition unit that included an IS*Apl1* element (Figure 1, panel A; online Technical Appendix Figure 1, panel A) flanked by the inverted repeats (IR-R1, IR-R2, and IR-L1). We verified the chromosomal location for the *mcr-1* gene by sequencing the genome to completion, using long-read single-molecule real-time sequencing (Pacific Biosciences, Menlo Park, CA, USA; online Technical Appendix Figure 2); the resulting contigs of *E. coli* RL465 were deposited in the European Nucleotide Archive under accession no. PRJEB14095. One copy of the IS*Apl1-mcr-1* transposition unit was located in the region between a predicted 4Fe-4S ferredoxin-type protein (*ydhY*) and *ldtE* (L,D-transpeptidase) (bp 2652307–2665241), and flanked on either side by a 2-bp direct repeat (CA). We observed a similar situation for the IS*Ecp1*-$bla_{\text{CTX-M-15}}$–*orf477* transposition unit (online Technical Appendix Figure 1, panel B). However, this insertion mapped to a different chromosomal location in a region encoding a defective lambdoid prophage inserted between the molybdate ABC transporter operon (*modABC*) and the biotin biosynthesis operon (*bioABCDF*) (bp 1662140–1716472). It was flanked by direct repeats (TGGTT).

We reexamined our collection of 424 genome sequenced ESBL- and carbapenemase-encoding *E. coli* isolates, obtained during 2010–2014 (*2*), for isolates that harbored $bla_{\text{CTX-M-15}}$ at a chromosomal location identical to that found in *E. coli* RL465. We detected 3 such isolates from 2010–2011 from companion animals and livestock (R107, sock swab dairy cattle farm, 2011; R208, sock swab pig fattening farm, 2011; V177, sick dog, 2010), and 11 consecutive isolates from a hemato-oncologic patient (*5*), obtained within an 11-month period during 2011–2012 (E006910, E007337, E007651, E007825, E000565, E002592, E002816, E003488, E005417, E006587, E006874) (Figure, panel B). All of these isolates were ST410 and negative for the *mcr-1* gene. Phylogenetic analysis of the core genome of these isolates with *E. coli* RL465 using the program Harvest Suite (*6*) indicated they were highly related and separated from *E. coli* V177 (the oldest isolate) by 66 (E006910, E007651) to 110 (E007337) single-nucleotide polymorphisms (core genome size 94%, representing 4.58 Mbp). Thus, our results suggest that transposition of the IS*Apl*1-*mcr-1* unit to the chromosome in *E. coli* RL465 is a later event and probably occurred after transfer of the $bla_{\text{CTX-M-15}}$ allele to the distinct chromosomal location into this *E. coli* ST410 subclone.

These findings highlight 2 independent points. First, our results extend data on the mobility of IS*Apl*1-*mcr-1* to a chromosomal location and reveal a new dimension in the transmissible nature of *mcr-1* in colistin-resistant *Enterobacteriaceae* isolates and their ecology. Second, clonal isolates of ST410 have been isolated from diverse environments, livestock, companion animals, and humans and, as we demonstrate here, in turkey hen meat (*7*,*8*). Thus, the simultaneous spread of the *mcr-1* and $bla_{\text{CTX-M-15}}$ genes mediated by a single bacterial clone is real and suggests that *mcr-1* is already present in the diverse reservoirs inhabited by these isolates.

**Acknowledgments**

We thank Christina Gerstmann, Natalia Lest, Simone Severitt, and Nicole Mrotzek for excellent technical assistance and Geovanna Brenner Michael, Yvonne Pfeifer, Axel Hamprecht, Lena Biehl, and Martin Kaase for providing the nonfood isolates.

This study was supported by grants to the German Center of Infection Research (DZIF), and the Zoonoses Network "ESBL and fluoroquinolone resistance in Enterobacteriaceae (RESET)" Consortium through the German Federal Ministry of Education and Research (BMBF; grant numbers 8000 701–3 [HZI], 01KI1013G and 01KI1313G to T.C. and C.I., and TI06.001 and 8032808811 to T.C.).

L.F., C.I., and T.C. conceived the study; S.E.W., K.G., H.G., S.D., B.B., C.S., and J.O. performed experiments; A.I., J.F., H.S., B.G., and A.K. contributed isolates and reagents; L.F., B.B., C.I., and T.C. analyzed the data; and T.C. and L.F. wrote the manuscript, which all authors approved.

B.G. is currently employed with the European Food Safety Authority (EFSA) in its BIOCONTAM Unit that provides scientific and administrative support to EFSA's scientific activities in the area of Microbial Risk Assessment. The positions and opinions presented in this article are those of the authors alone and are not intended to represent the views or scientific works of EFSA. The other authors have nothing to proclaim.

### References


1. Liu Y-Y, Wang Y, Walsh TR, Yi L-X, Zhang R, Spencer J, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis. 2016;16:161–8. http://dx.doi.org/10.1016/S1473-3099(15)00424-7
2. Falgenhauer L, Waezsada S-E, Yao Y, Imirzalioglu C, Käsbohrer A, Roesler U, et al.; RESET Consortium. Colistin resistance gene *mcr-1* in extended-spectrum b-lactamase-producing and carbapenemase-producing gram-negative bacteria in Germany. Lancet Infect Dis. 2016;16:282–3. http://dx.doi.org/10.1016/S1473-3099(16)00009-8
3. Hasman H, Hammerum AM, Hansen F, Hendriksen RS, Olesen B, Agersø Y, et al. Detection of *mcr-1* encoding plasmid-mediated colistin-resistant *Escherichia coli* isolates from human bloodstream infection and imported chicken meat, Denmark 2015. Euro Surveill. 2015;20:30085. http://dx.doi.org/10.2807/1560-7917.ES.2015.20.49.30085
4. Skov RL, Monnet DL. Plasmid-mediated colistin resistance (*mcr-1* gene): three months later, the story unfolds. Euro Surveill. 2016;21:1–6. http://dx.doi.org/10.2807/1560-7917.ES.2016.21.9.30155
5. Vehreschild MJGT, Hamprecht A, Peterson L, Schubert S, Häntschel M, Peter S, et al. A multicentre cohort study on colonization and infection with ESBL-producing *Enterobacteriaceae* in high-risk patients with haematological malignancies. J Antimicrob Chemother. 2014;69:3387–92. http://dx.doi.org/10.1093/jac/dku305
6. Treangen TJ, Ondov BD, Koren S, Phillippy AM. The Harvest suite for rapid core-genome alignment and visualization of thousands of intraspecific microbial genomes. Genome Biol. 2014;15:524. http://dx.doi.org/10.1186/s13059-014-0524-x
7. Falgenhauer L, Imirzalioglu C, Ghosh H, Gwozdzinski K, Schmiedel J, Gentil K, et al. Circulation of clonal populations of fluoroquinolone-resistant CTX-M-15-producing *Escherichia coli* ST410 in humans and animals in Germany. Int J Antimicrob Agents. 2016;47:457–65. http://dx.doi.org/10.1016/j.ijantimicag.2016.03.019
8. Schaufler K, Semmler T, Wieler LH, Wöhrmann M, Baddam R, Ahmed N, et al. Clonal spread and interspecies transmission of clinically relevant ESBL-producing *Escherichia coli* of ST410—another successful pandemic clone? FEMS Microbiol Ecol. 2016;92:fiv155. http://dx.doi.org/10.1093/femsec/fiv155


Address for correspondence: Trinad Chakraborty, Institute of Medical Microbiology, Schubertstrasse 81, 35392 Giessen, Germany; email: Trinad.Chakraborty@mikrobio.med.uni-giessen.de

# Specificity of Dengue NS1 Antigen in Differential Diagnosis of Dengue and Zika Virus Infection

**Séverine Matheus, Rachida Boukhari, Bhety Labeau, Valérie Ernault, Laetitia Bremand, Mirdad Kazanji, Dominique Rousset**

Author affiliations: Institut Pasteur de la Guyane, Cayenne, French Guiana (S. Matheus, B. Labeau, L. Bremand, M. Kazanji, D. Rousset); Centre Hospitalier de l'Ouest-Guyanais, Saint-Laurent du Maroni, French Guiana (R. Boukhari, V. Ernault)

DOI: http://dx.doi.org/10.3201/eid2209.160725

**To the Editor:** Circulation of new arboviruses of the genus *Flavivirus* poses a major problem for differential diagnosis. Zika virus, a mosquitoborne virus of the family *Flaviviridae*, is closely related to other arboviruses circulating in the Americas, including dengue, yellow fever, Saint Louis encephalitis, and West Nile viruses (*1*,*2*). Serologic cross-reactivity between these arboviruses is common; thus, to ensure optimal patient care and accurate epidemiologic surveillance, an effective differential diagnosis is required in regions with active transmission of dengue virus and circulation of Zika virus (*2–4*).

Cross-reactivity between flaviviruses has been reported in antibody assays and in tests for Dengue nonstructural 1 glycoprotein (NS1) antigen. Gyurech et al. (*5*) reported false-positive test results for dengue NS1 antigen in a patient with acute Zika virus infection. Of the 3 NS1 tests used in that study, only the SD Bioline Dengue Duo (Standard Diagnostics, Inc., Gyeonggi-do, South Korea) showed positive results for 3 of 4 sequential serum samples from the patient.

Cross-reactivity in NS1 dengue tests (ELISA and immunochromatographic) using serum samples from patients with acute Zika virus infection would have medically significant consequences. We therefore conducted a retrospective analysis of the differential diagnosis for dengue and Zika virus infections since the beginning of the Zika virus outbreak in French Guiana, a department of France on the northeast coast of South America.

French Guiana is subject to endemoepidemic circulation of dengue and experienced a large outbreak of chikungunya in 2014. We conducted our study from December 17, 2015 (the time of biologic confirmation of the first case of Zika virus disease in French Guiana), through March 2, 2016. During that time, the incidence of dengue virus infection in French Guiana was low, and only 1 sporadic case was confirmed. We studied clinical samples collected during this period from all patients with suspected arbovirus infection.

Article DOI: http://dx.doi.org/10.3201/eid2209.160692

# Chromosomal Locations of *mcr-1* and *bla*$_{\text{CTX-M-15}}$ in Fluoroquinolone-Resistant *Escherichia coli* ST410

## Technical Appendix

### Collection of Isolates

Retail food samples originating from cattle, swine, and poultry (meat and milk) were sampled by food inspectors in 4 different regions of Germany during May 2012–April 2013. All samples were unrelated to each other. No information was recorded about the country from which the animals originated. Extended-spectrum β-lactamases (ESBL)–producing isolates were selected by using MacConkey agar plates supplemented with 1 mg/L cefotaxime. A subset of 62 *Escherichia coli* isolates was analyzed by using whole-genome sequencing.

### Antimicrobial Susceptibility Testing

Antimicrobial susceptibility tests were performed by using VITEK 2 (AST-card: N248; bioMérieux, Nürtingen, Germany). Colistin susceptibility testing was performed by using broth microdilution according to European Union Commission Implementing Decision 2013/652/EU (http://eur-lex.europa.eu/legal-content/EN/TXT/?uri = CELEX:32013D0652) (using EUVSEC Sensititer plates, Trek Diagnostic systems, Thermo Fischer Scientific, Dreieich, Germany). The results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (2015) and Clinical and Laboratory Standards Institute (2015) guidelines (*1*,*2*).

### Whole-Genome Sequencing

Whole-genome DNA was isolated from overnight cultures by using the Purelink Genome DNA Mini kit (Invitrogen, Darmstadt, Germany). The sequencing library was produced by using Illumina Nextera XT Kit and sequenced on a MiSeq instrument (Illumina, San Diego, CA,

USA), with 2 × 300 read length. The average read length accounted for 180 nt with an average coverage of 51×. Raw reads were assembled by using SPAdes (v. 3.0) (*3*). To confirm the chromosomal location of *mcr-1* in *E. coli* RL465, long-read single-molecule real-time (SMRT) sequencing was performed. For this, DNA was isolated by using the method described by Pitcher et al. (*4*). A SMRTbell template library was prepared according to the instructions, following the Procedure & Checklist—10 kb Template Preparation Using BluePippin Size-Selection System. Briefly, for preparation of 15-kb libraries 8 μg genomic DNA was sheared by using g-tubes (Covaris, Woburn, MA, USA) according to the manufacturer´s instructions. DNA was end-repaired and ligated overnight to hairpin adapters applying components from the DNA/Polymerase Binding Kit P6 (Pacific Biosciences, Menlo Park, CA, USA). Reactions were conducted according to the manufacturer´s instructions. BluePippin Size-Selection to 4 kb was performed according to the manufacturer´s instructions (Sage Science, Beverly, MA, USA). Conditions for annealing of sequencing primers and binding of polymerase to purified SMRTbell template were assessed with the Calculator in RS Remote (Pacific Biosciences). SMRT sequencing was conducted on the PacBio RSII (Pacific Biosciences) taking one 240-min movie for a single SMRT cell. We assembled PacBio reads using RS_ HGAP_ Assembly.3 protocol included in the SMRT Portal 2.3.0. The number of reads from PacBio sequencing accounted for 93,593 with a mean read length of 12,057 nt. To obtain a high-quality genome sequence, we mapped paired-end reads from Illumina sequencing using Burrows–Wheeler Aligner (*5*). The chromosome displayed a size of 4,894,900 bp (167× coverage). One plasmid of 157,187 bp (57× coverage), and 2 phage-like elements 89,746 bp, (Element 1, 39× coverage, circular), and 61,544 bp (Element 2, 234× coverage, linear) were detected.

## In Silico Analyses

We identified resistance genes using ResFinder (*6*), virulence genes using VirulenceFinder (*7*), plasmid incompatibility groups and plasmid multilocus sequence typing with PlasmidFinder and pMLST (*8*), and multilocus sequence types using MLST 1.8, according to the scheme of Wirth et al. (*9*,*10*). The genetic environment of *mcr-1* and $bla_{\text{CTX-M-15}}$ was identified using blastn and ISFinder (*11*,*12*). Annotation of the *E. coli* RL465 genome and extrachromosomal units was performed using RAST (*13*). To identify phages, we used the program PHAST (*14*).

## Conjugation Experiments

We conducted conjugation experiments at 37°C or at ambient temperatures as described previously (*15*) using *E. coli* J53 *Az*$^{r}$ as a recipient and 2 mg/L colistinsulfate and 200 mg/L sodiumazide as selective agents. Replicon typing of the transconjugants was performed as described in the literature (*16*,*17*).

### References


1. European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 5.0, 2015 [cited 2015 May 3]. http://www.eucast.org

2. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Twenty-fifth informational supplement. CLSI document M100–S25. Wayne (PA): The Institute; 2015.

3. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol. 2012;19:455–77. PubMed http://dx.doi.org/10.1089/cmb.2012.0021

4. Pitcher DG, Saunders NA, Owen RJ. Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. Lett Appl Microbiol. 1989;8:151–6. http://dx.doi.org/10.1111/j.1472-765X.1989.tb00262.x.

5. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009;25:1754–60. PubMed http://dx.doi.org/10.1093/bioinformatics/btp324

6. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, et al. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother. 2012;67:2640–4. PubMed http://dx.doi.org/10.1093/jac/dks261

7. Joensen KG, Scheutz F, Lund O, Hasman H, Kaas RS, Nielsen EM, et al. Real-time whole-genome sequencing for routine typing, surveillance, and outbreak detection of verotoxigenic *Escherichia coli*. J Clin Microbiol. 2014;52:1501–10. PubMed http://dx.doi.org/10.1128/JCM.03617-13

8. Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, Villa L, et al. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother. 2014;58:3895–903. PubMed http://dx.doi.org/10.1128/AAC.02412-14

9. Zankari E, Hasman H, Kaas RS, Seyfarth AM, Agersø Y, Lund O, et al. Genotyping using whole-genome sequencing is a realistic alternative to surveillance based on phenotypic antimicrobial susceptibility testing. J Antimicrob Chemother. 2013;68:771–7. PubMed http://dx.doi.org/10.1093/jac/dks496

10. Wirth T, Falush D, Lan R, Colles F, Mensa P, Wieler LH, et al. Sex and virulence in *Escherichia coli*: an evolutionary perspective. Mol Microbiol. 2006;60:1136–51. PubMed http://dx.doi.org/10.1111/j.1365-2958.2006.05172.x

11. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990;215:403–10. PubMed http://dx.doi.org/10.1016/S0022-2836(05)80360-2

12. Siguier P, Perochon J, Lestrade L, Mahillon J, Chandler M. ISfinder: the reference centre for bacterial insertion sequences. Nucleic Acids Res. 2006;34:D32–6. PubMed http://dx.doi.org/10.1093/nar/gkj014

13. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, et al. The RAST Server: rapid annotations using subsystems technology. BMC Genomics. 2008;9:75. PubMed http://dx.doi.org/10.1186/1471-2164-9-75

14. Zhou Y, Liang Y, Lynch KH, Dennis JJ, Wishart DS. PHAST: a fast phage search tool. Nucleic Acids Res. 2011;39(suppl):W347-5. PubMed2 http://dx.doi.org/10.1093/nar/gkr485

15. Falgenhauer L, Waezsada S-E, Yao Y, Imirzalioglu C, Käsbohrer A, Roesler U, et al.; RESET consortium. Colistin resistance gene *mcr-1* in extended-spectrum β-lactamase-producing and carbapenemase-producing gram-negative bacteria in Germany. Lancet Infect Dis. 2016;16:282–3. PubMed http://dx.doi.org/10.1016/S1473-3099(16)00009-8

16. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. Identification of plasmids by PCR-based replicon typing. J Microbiol Methods. 2005;63:219–28. PubMed http://dx.doi.org/10.1016/j.mimet.2005.03.018

17. Johnson TJ, Bielak EM, Fortini D, Hansen LH, Hasman H, Debroy C, et al. Expansion of the IncX plasmid family for improved identification and typing of novel plasmids in drug-resistant *Enterobacteriaceae*. Plasmid. 2012;68:43–50. PubMed http://dx.doi.org/10.1016/j.plasmid.2012.03.001

**Technical Appendix Table 1.** Depiction of the MIC of the *mcr-1*-encoding and extended-spectrum β-lactamase ESBL–producing isolates from retail food*

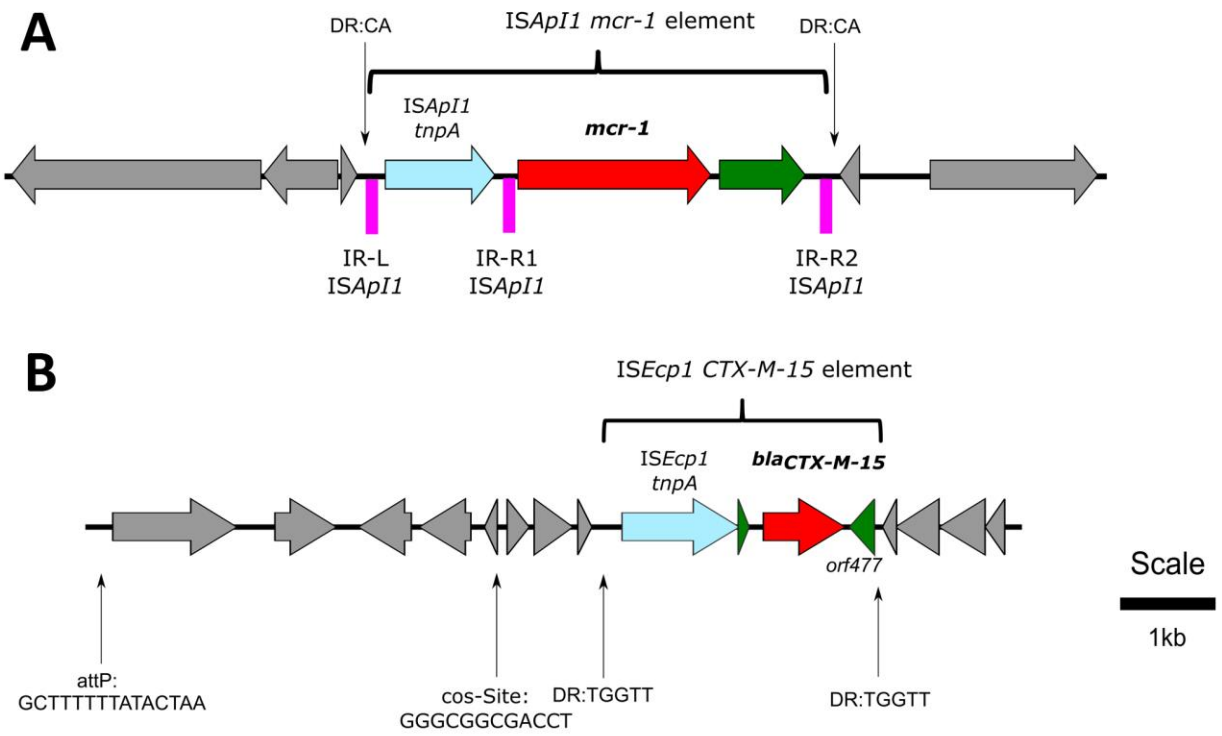
| Antimicrobial drug class, drug | RL138 | | RL145 | | RL158 | | RL465 | |
|---|---|---|---|---|---|---|---|---|
| | MIC | Interpretation | MIC | Interpretation | MIC | Interpretation | MIC | Interpretation |
| Aminoglycosides | | | | | | | | |
| Amikacin | ≤2 | S | ≤2 | S | ≤2 | S | ≤2 | S |
| Gentamicin | ≤1 | S | ≤1 | S | ≤1 | S | ≤1 | S |
| Tobramycin | ≤1 | S | ≤1 | S | ≤1 | S | ≤1 | S |
| Penicillins | | | | | | | | |
| Ampicillin | ≥32 | R | ≥32 | R | ≥32 | R | ≥32 | R |
| Ampicillin/Sulbactam | 16 | R | ≥32 | R | 16 | R | 16 | R |
| Piperacillin | ≥128 | R | ≥128 | R | ≥128 | R | ≥128 | R |
| Piperacillin/Tazobactam | ≤4 | S | 8 | S | ≤4 | S | ≤4 | S |
| Carbapenems | | | | | | | | |
| Ertapenem | ≤0.5 | S | ≤0.5 | S | ≤0.5 | S | ≤0.5 | S |
| Imipenem | ≤0.25 | S | ≤0.25 | S | ≤0.25 | S | ≤0.25 | S |
| Meropenem | ≤0.25 | S | ≤0.25 | S | ≤0.25 | S | ≤0.25 | S |
| Cephalosporins | | | | | | | | |
| Cefepime | 2 | I | ≤1 | S | 2 | I | 2 | I |
| Cefotaxime | 8 | R | 8 | R | 8 | R | 32 | R |
| Cefpodoxime | ≥8 | R | ≥8 | R | ≥8 | R | ≥8 | R |
| Ceftazidime | ≤1 | S | 16 | R | ≤1 | S | 4 | R |
| Cefuroxime | ≥64 | R | ≥64 | R | ≥64 | R | ≥64 | R |
| Fluoroquinolones | | | | | | | | |
| Ciprofloxacin | ≥4 | R | ≤0.25 | S | ≤0.25 | S | ≥4 | R |
| Moxifloxacin | ≥8 | R | ≤0.25 | S | ≤0.25 | S | ≥8 | R |
| Miscellaneous agents | | | | | | | | |
| Fosfomycin | ≤16 | S | ≤16 | S | ≤16 | S | ≤16 | S |
| Trimethoprim/ sulfamethoxazole | ≥320 | R | ≤20 | S | ≥320 | R | ≥320 | R |
| Monobactams: Aztreonam | ≤1 | S | 2 | I | 2 | I | 16 | R |
| Tetracyclines | | | | | | | | |
| Tetracycline | ≥16 | R | ≥16 | R | ≤1 | R | ≥16 | R |
| Tigecycline | ≤0.5 | S | ≤0.5 | S | ≤0.5 | S | ≤0.5 | S |

*The MIC results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (2015) and the Clinical and Laboratory Standards Institute (2015) guidelines (*1,2*). MIC, minimal inhibitory concentration; R, resistant; S, susceptible.

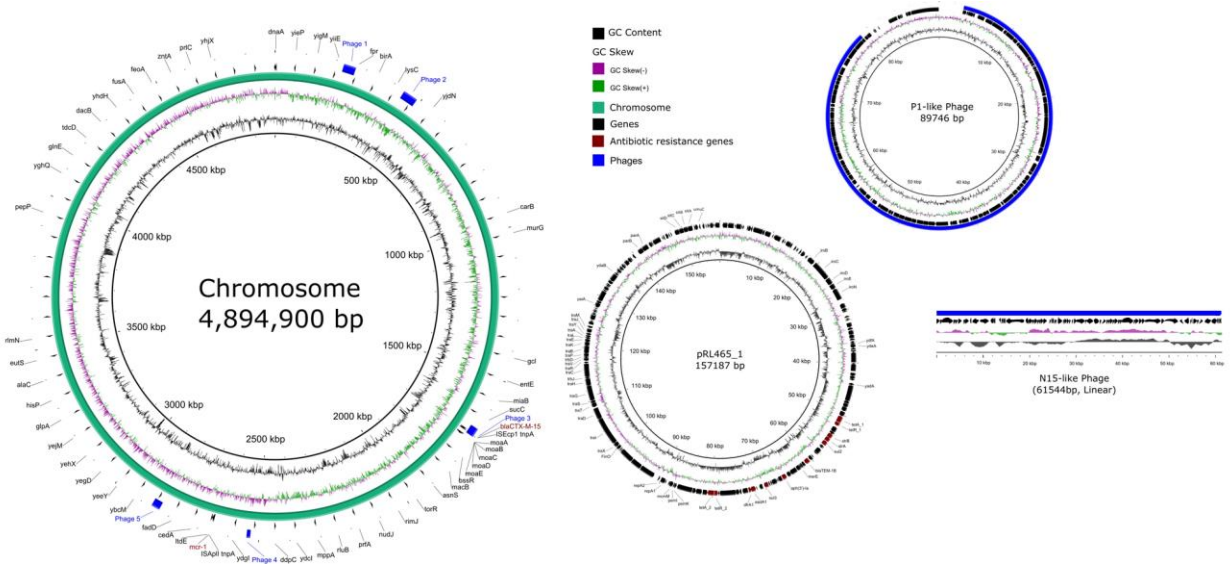
**Technical Appendix Table 2.** Characteristics of the *mcr-1*-encoding ESBL-producing *Escherichia coli* isolates from retail meat*

| Variable | Characteristic | | | |
|---|---|---|---|---|
| Name | RL138 | RL145 | RL158 | RL465 |
| Species | *E. coli* | *E. coli* | *E. coli* | *E. coli* |
| Sequence type | ST602 | ST88 | ST357 | ST410 |
| Year of isolation | 2012 | 2012 | 2012 | 2013 |
| Colistin MIC | 4 mg/L | 4 mg/L | 4 mg/L | 4 mg/L |
| Source | Chicken breast | Minced meat, beef | Turkey hen Schnitzel | Turkey hen meat |
| Antimicrobial resistance genes | *aadA1, aadA2-like, $bla_{CTX-M-14}$, catA1-like, cmlA1-like, dfrA1, mcr-1, strA-like, strB, sul1, sul3, tet(A)* | *aadA1-like, aph(3′)-Ia-like, $bla_{OXA-1}$, $bla_{TEM-52C}$, mcr-1, sul1, tet(B)* | *aadA1, $bla_{CTX-M-1}$, dfrA1, mcr-1, sul1-like, sul2* | *aadA1*, *aph(3′)-Ia*, *$bla_{CTX-M-15}$*, *$bla_{TEM-1B}$*, *dfrA1*, *mcr-1, strA-like, strB-like, sul2, sul3, tet(A)-like* |
| Virulence genes | *iroN, iss, lpfA, mchF, tsh* | *iha, iroN, iss, lpfA, mchF, mcmA* | *iroN, iss, mchF* | *cma, iroN, iss, lpfA* |
| Plasmid incompatibility groups | IncHI2, IncFIC(FII), IncHI2A, IncI1, IncFIB(AP001918), IncFIA, IncP, Col(MG828) | IncFIB(AP001918), IncI1, IncFIC(FII), IncI2, IncX4, ColRNAI | IncFIC(FII), IncFIA, IncI1, IncFIB(AP001918), ColRNAI, IncX4, Col(MG828) | IncFIB(AP001918), IncFII(pCoo), Col156, ColRNAI |
| pMLST | IncHI2[ST-4], IncI1(unknown sequence type), IncF[F18:A6*:B1] | IncI1[ST-36], IncF[F18:A-:B1] | IncF[F18:A6*:B1], IncI1[ST-7] | IncF [F16:A-:B1] |
| *mcr-1* location | IncHI2 plasmid | IncX4 plasmid | IncX4 plasmid | Chromosome |

*MIC, minimal inhibitory concentration; pMLST, plasmid multilocus sequence type.




**Technical Appendix Figure 1.** Genetic environments of the chromosomally located antimicrobial resistance genes (A) *mcr-1* and (B) *bla*$_{\text{CTX-M-15}}$ of the *Escherichia coli* isolate RL465. *attR*, right phage attachment site; DR, direct repeat; cos site, cohesive end sequence of prophage; IR, inverted repeats. Genes marked in red display an antimicrobial resistance gene; in light blue transposase genes from the transposition units, green, other genes in the transposition units, pink bars depict the presence of the inverted repeats of IS*ApI1.* Unrelated flanking genes are shaded gray.




**Technical Appendix Figure 2.** Schematic depiction of the chromosome, its IncFII/FIB plasmid and the 2 phage elements in *Escherichia coli* RL465.